STREAMLINE® Scale Up

Application Note

Expanded bed adsorption

Key words: STREAMLINE, yeast, scale up, automation, sanitization.

Abstract

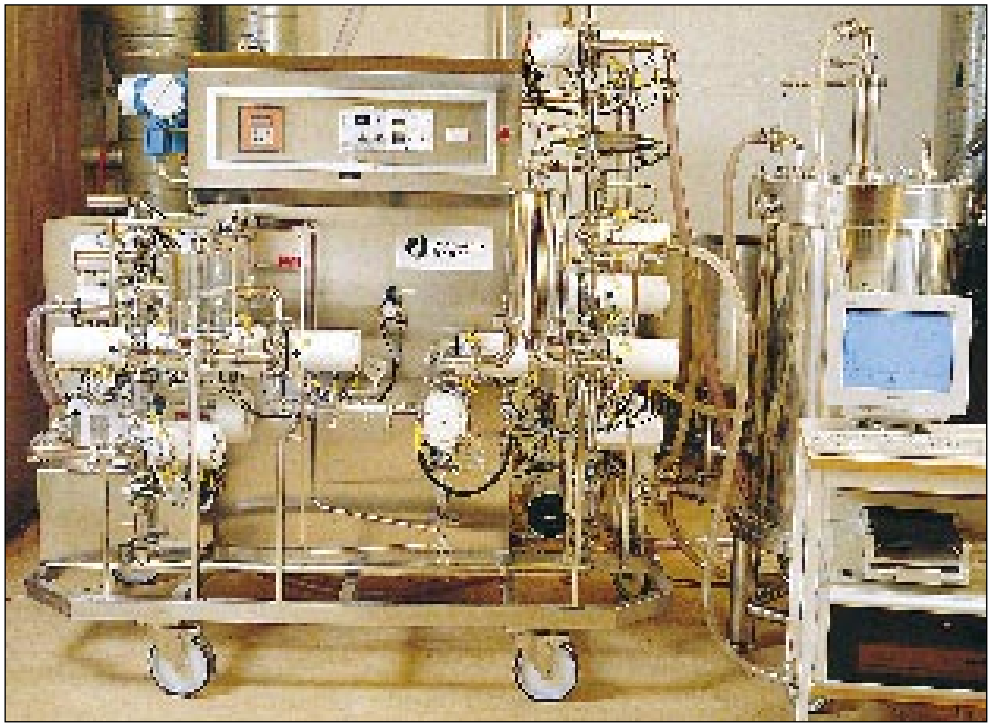
This application note describes the scale up of an expanded bed adsorption process using STREAMLINE DEAE chromatography adsorbent. Method development work was performed at laboratory scale on STREAMLINE 25 column (25 mm i.d.) run on BioPilot® System. The established process was then verified in a pilot scale set-up using STREAMLINE 200 column (200 mm i.d.), before final scale up to a production scale, fully automated capture step, on STREAMLINE 600 column (600 mm i.d.) and a specially designed STREAMLINE system controlled by UNICORN® control system. At production scale, 440 litres of a yeast suspension were processed in a single pass operation on a column with a sedimented bed volume of 42 litres.

At each scale, the hydrodynamic and functional properties of the expanded bed were verified by residence time distribution tests and determination of breakthrough capacity.

The hygienic status of the STREAMLINE column and system used at production scale was evaluated by performing a sanitization study in which the column and system were challenged with culture broths of yeast and bacteria.

Introduction

Expanded bed adsorption is a unique technique offering advantages in the recovery of proteins from crude fermentation broths. It combines the process steps clarification, concentration and capture into one unit operation, and thereby improves process economy by decreasing the number of processing steps, increasing yield, shortening overall processing time and reducing processing costs.



A production scale STREAMLINE system and column.

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Expanded bed adsorption is rapidly gaining recognition as a viable tool for chromatography in the initial phase of downstream processing and many such processes are finding their way into the production hall.

The purpose of this study was to demonstrate the scalability of STREAMLINE expanded bed adsorption by defining a realistic process example at small scale and taking it all the way up to a fully automated process scale unit operation.

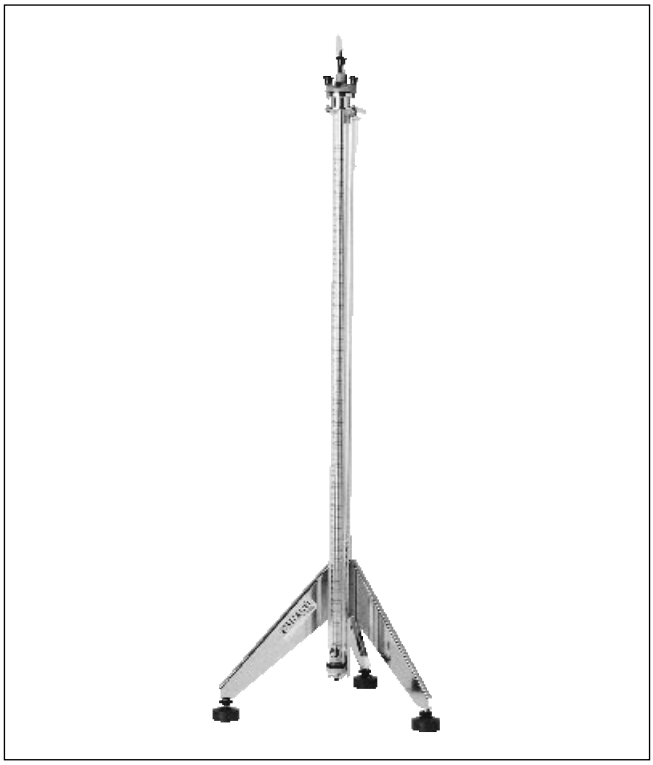
Materials and methods

The feed material used in this study was based on BSA (Sigma, code no 9048-46-8) spiked into a suspension of baker's yeast. The feed was prepared by making a buffer concentrate containing all ingredients except for the yeast. Within 1 to 2 hours before sample application a 20–25% yeast suspension in water was added to the buffer concentrate. The final composition of the feed was: 20 mM Tris, 50 mM NaCl, 4–5% yeast (dry weight), 2.0 mg BSA/ml. Conductivity was 5–6 mS/cm and pH was adjusted to pH 7.5.

Expansion, equilibration, sample application and wash were performed at a linear flow rate of 300 cm/h using 20 mM Tris, pH 7.5 (buffer A). Elution was performed by a single step procedure using a solution of 1.0 M NaCl in buffer A. Elution was performed in packed bed mode, using downward flow, at a linear flow rate of 100 cm/h. The amount of STREAMLINE DEAE adsorbent used at each scale corresponded to a sedimented bed height of 15 cm.

Processing parameters were consistent throughout the different scales.

Cleaning-in-place (CIP) and sanitization-in-place (SIP) were performed immediately after the elution step, according to the protocol outlined in Table 1, using upward flow in expanded bed mode. The same protocol was applied at all three scales. The cleaning step with 0.5 M NaOH/1.0 M NaCl included a pause to increase exposure time to between 4 and 15 hours.



STREAMLINE 25 column.

Table 1. Protocol for cleaning-in-place and sanitization-in-place.

CIP solution	Flow rate (cm/h)	Volume (sedimented bed volumes)
2 M NaCl	100	3
distilled water	100	3
0.5 M NaOH/1.0 M NaCl	100	6
distilled water	100	3
20% ethanol/10% HAc	100	3

Equipment

Laboratory scale

The column used was STREAMLINE 25, a glass column with an inner diameter of 25 mm giving a sedimented bed volume of 74 ml at a sedimented bed height of 15 cm. It was equipped with a hydraulically adjustable adaptor. The column was connected in line to a BioPilot System controlled by UNICORN. The hydraulic chamber of the column was connected to the C pump on BioPilot System. The adaptor was raised by manually disconnecting the C pump, and blocking the column outlet while pumping buffer with upward flow through the bed.

STREAMLINE 200 column connected to a manual system.

Pilot scale

The column used was STREAMLINE 200, a glass column with an inner diameter of 200 mm giving a sedimented bed volume of 4.7 litres at a sedimented bed height of 15 cm. The column has a hydraulically adjustable adaptor. It was connected to a manual system consisting of manual valves, two peristaltic pumps, and a UV-1 monitor equipped with an industrial flow cell and connected to a chart recorder.

Production scale

The column used was STREAMLINE 600, a stainless steel column with an inner diameter of 600 mm giving a sedimented bed volume of 42 litres at a sedimented bed height of 15 cm. The column has a hydraulically adjustable adaptor and an adsorbent sensor mounted on the adaptor end-plate. The adaptor is lowered by pumping hydraulic liquid into the hydraulic chamber and raised by the upward liquid flow through the expanded bed. The adsorbent sensor uses ultrasonic detection and is specially designed to detect the presence of STREAMLINE adsorbent beads: it can discriminate between beads and other types of particulate material such as cells and cell debris originating from the feed material. The adsorbent sensor can thus be used to keep the adaptor close to the expanded bed surface throughout the wash cycle subsequent to sample application. The column also has an adaptor position indicator which provides continuous information about the adaptor during the process cycle.

The system was specially designed for the purpose of evaluating the functionality of a completely automated expanded bed adsorption process using STREAMLINE 600 column. The chromatographic

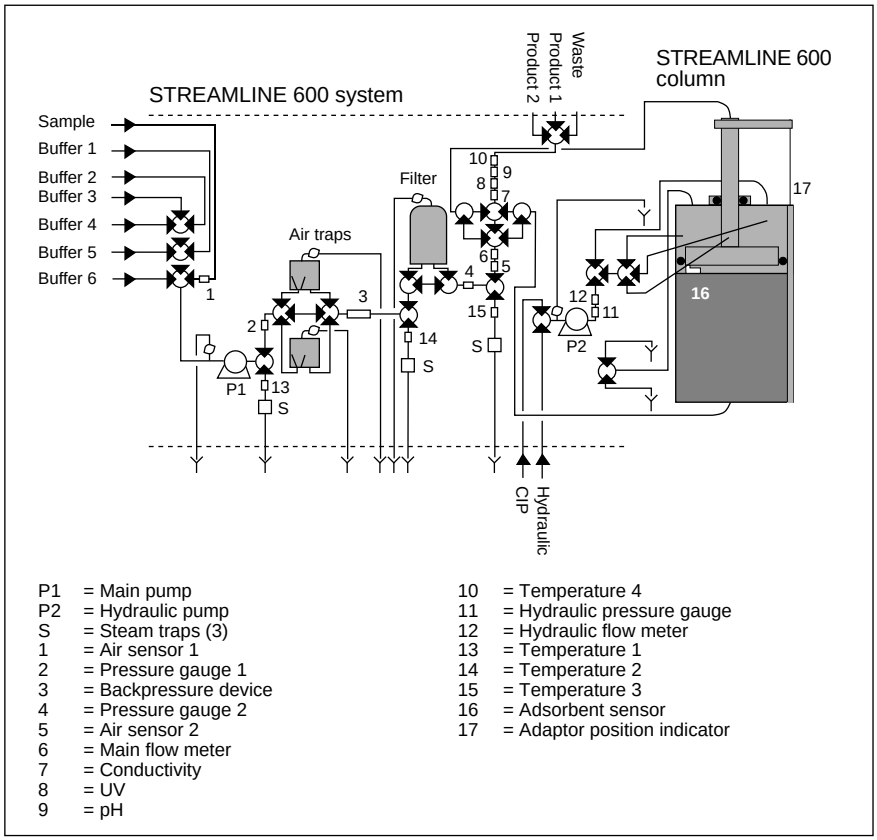


Fig. 1. STREAMLINE column and system for production scale operation.

part consists of seven inlets, one pump, two air traps (one for feed application), one filter, detectors for UV, conductivity, pH and temperature, and three outlets. The hydraulic part consists of two inlets, one for hydraulic liquid, one for CIP solution; one hydraulic pump; and one inlet and one outlet on top of the hydraulic chamber of the column (the compartment above the adaptor plate). For a full description of the system configuration, see Fig. 1. The upper part of the column has three extra inlets to the hydraulic chamber through the column tube. These inlets are used during the CIP/SIP of the hydraulic chamber to improve the efficiency of the wash. The system was connected to UNICORN which provided complete automated processing.

Verification of bed stability

The stability of the expanded bed is critical for optimal performance and must be consistent from scale to scale to allow reproducible processing. In this study a Residence Time Distribution (RTD) test was applied to verify that the equipment used at each scale yielded a stable expanded bed with a minimum of axial dispersion. The RTD test is a tracer stimulus method that can be used to assess the degree of back-mixing (axial dispersion) in an expanded bed by defining the number of theoretical plates. The RTD test was also performed on STREAMLINE 50, a 50 mm i.d. column, and STREAMLINE 1200, a 1200 mm i.d. column.

Buffer was pumped through the bed at a flow rate of 300 cm/h until the bed was fully expanded. The adaptor was then lowered until there was about 0.5–1 cm between the net and the expanded bed surface. When the UV-signal showed a stable baseline, a buffer-acetone mixture (0.25% v/v) was pumped through the expanded bed until the UV-signal stabilized at maximum absorbance (Fig. 2). Then buffer was pumped through the expanded bed again until the UV-signal stabilized at the baseline level (Fig. 2). The number of theoretical plates was calculated from the negative step input signal by using the formula:

$$N = t^2 / \sigma^2$$

where

t = mean residence time
σ = standard deviation

t was taken as the distance from the start of wash out of the buffer-acetone mixture (“mark” in Fig. 2) to 50% of the maximum UV-signal.

σ was measured as half the distance between the points 15.85% and 84.15% of the maximum UV-signal (Fig. 2).

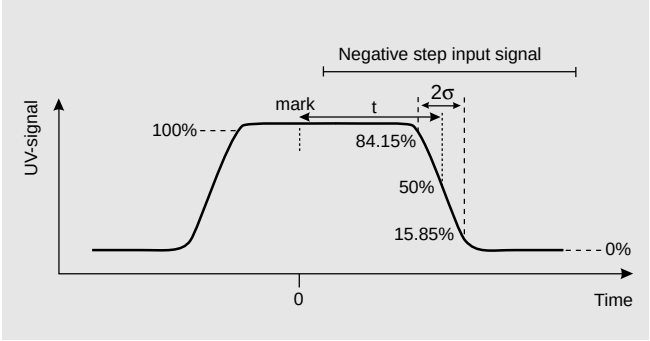


Fig. 2. UV-signal recording during the test procedure for the determination of the number of theoretical plates.

The results from the RTD testing are shown in Table 2. From our experience, 30 theoretical plates is considered an acceptable level at a sedimented bed height of 15 cm. As can be seen from Table 2, a drop in N was observed up to a column diameter of 200 mm, but data were consistent from 200 mm up to 1200 mm i.d.

Table 2. Number of theoretical plates for the different column sizes as determined by RTD testing.

Column	Number of theoretical plates (N)
STREAMLINE 25	60–70
STREAMLINE 50	50–60
STREAMLINE 200	40–50
STREAMLINE 600	40–50
STREAMLINE 1200	40–50

Breakthrough capacity

The RTD test described above provides a good reference for production scale system design. Extensive back-mixing in an expanded bed will lead to poor contacting efficiency between the solute and the binding groups on the adsorbent. The RTD test is a measure of the back-mixing and therefore a good indicator of the protein adsorption characteristics of the system. Determination of breakthrough capacity will however provide a more accurate measure of total function, i.e. of the hydrodynamic properties of the expanded bed and of the impact of these on the protein binding capacity–in other words, the chromatographic function of the system. Breakthrough capacity was determined at each scale to verify that the process equipment yielded a stable expanded bed, providing consistency in breakthrough capacity from scale to scale. The study also included STREAMLINE 50 column and a larger column, STREAMLINE 1200.

The sample applied for the breakthrough capacity determination was BSA (2 mg/ml) in 50 mM Tris pH 7.5. Sample was applied to the expanded bed at a linear flow rate of 300 cm/h. Concentration of BSA in the flow through (C) was determined by the UV-signal from an in-line UV monitor. Concentration of BSA at the column inlet (C_o) was determined by the UV-signal from the feed applied to the column. Applied BSA per ml sedimented adsorbent was calculated from the volume of feed applied after correction for the dead volume in system and expanded bed.

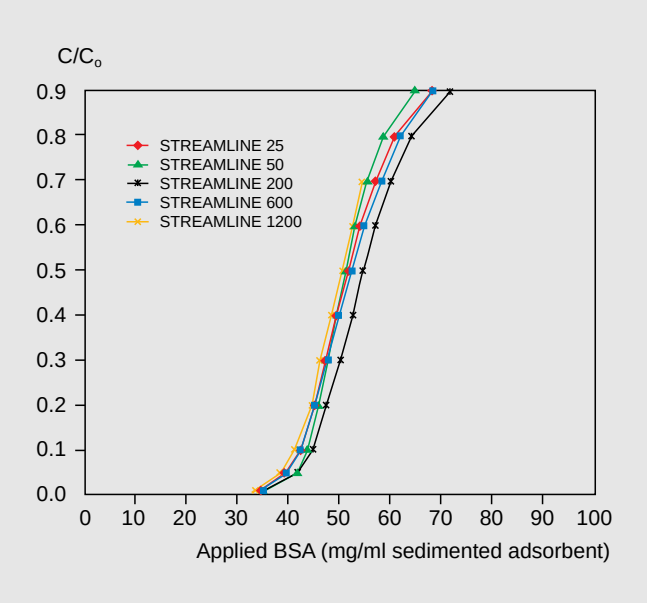


Fig. 3. Breakthrough curves for BSA on STREAMLINE DEAE from laboratory to production scale.

The results from the breakthrough capacity determination are shown in Fig. 3. The curves show good agreement between scales with respect to both breakthrough capacity and steepness of the curve. The results indicate complete scalability from STREAMLINE 25 with a sedimented bed volume of 74 ml up to STREAMLINE 1200 with a sedimented bed volume of 170 litres.

Expanded bed adsorption
STREAMLINE 25 column

Two separate runs were performed on STREAMLINE 25 column. STREAMLINE DEAE was expanded and equilibrated by running 10 sedimented bed volumes of buffer A through the bed. The bed expanded to 42.5 cm. Since the application of a crude feed causes the bed to expand further, the adaptor was positioned at a height of 83 cm during feed application. The flow was reversed a few times during sample application to prevent any clogging of the column nets or build-up of adsorbent under the adaptor end-plate. A feed volume corresponding to approximately 10 sedimented bed volumes was applied to the column followed by a wash with buffer A to remove non-bound substances and cell debris from the bed. The adaptor was lowered as soon as the feed had passed through the expanded bed. Wash buffer was applied until a stable base line was obtained. Flow was then stopped, the adsorbent allowed to sediment and the adaptor adjusted to the top of the sedimented bed. The packed bed was then washed with buffer A followed by elution of the product with buffer B. Finally, the CIP/SIP procedure was performed automatically overnight with the adaptor positioned at a height of 35 cm.

A chromatogram from the second run is shown in Fig. 4.

Results from the runs on STREAMLINE 25 are summarized in Table 3.

Table 3. Summary of process data from laboratory to production scale.

Column	Run ID	Sample volume (l)	Dry weight (%)	Load (g BSA/l sedimented adsorbent)	Wash vol. (SBV)	Peak vol. (SBV)	Yield (%)
STREAMLINE 25:	run 1	0.74	4.71	19.7	12	1.1	90
	run 2	0.75	4.83	19.9	11	1.2	87
STREAMLINE 200	run 1	48	4.82	20.4	16	1.9	88
STREAMLINE 600:	run 1	420	4.87	19.9	17	2.2	87
	run 2	440	4.48	20.8	11	2.1	92

SBV = sedimented bed volumes

STREAMLINE 200 column

One run was performed on STREAMLINE 200 column. The adsorbent was expanded and equilibrated by running 10 sedimented bed volumes of buffer A through the bed. The bed expanded to 42 cm. The adaptor was positioned at a height of 80 cm during feed application. Flow was reversed a few times to prevent clogging of the nets and packing of adsorbent beads under the adaptor end-plate. A feed volume corresponding to approximately 10 sedimented bed volumes was applied followed by a wash with buffer A. During the wash, the adaptor was lowered to a height of 57 cm when the descending UV-signal reached a value of 2.0 AU. The wash continued until the UV-signal was below 0.1 AU at which time the flow was stopped, the bed allowed to sediment and the adaptor adjusted to the top of the sedimented bed. The packed bed was then washed with buffer A followed by elution of the product with buffer B. CIP/SIP was performed as previously described for the STREAMLINE 25 column.

A chromatogram from the run on STREAMLINE 200 is shown in Fig. 5.

Results from the run on STREAMLINE 200 are summarized in Table 3.

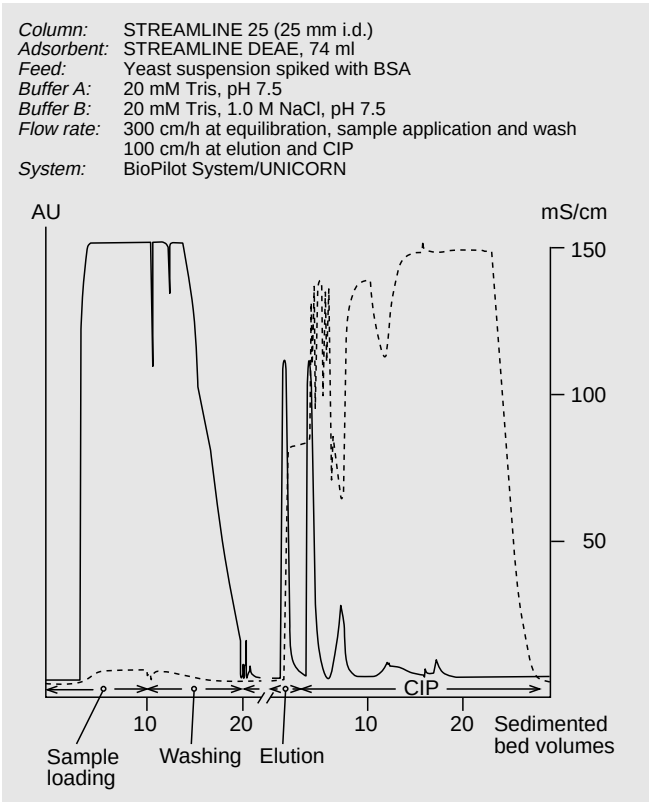


Fig. 4. Capture of BSA on STREAMLINE DEAE at laboratory scale.

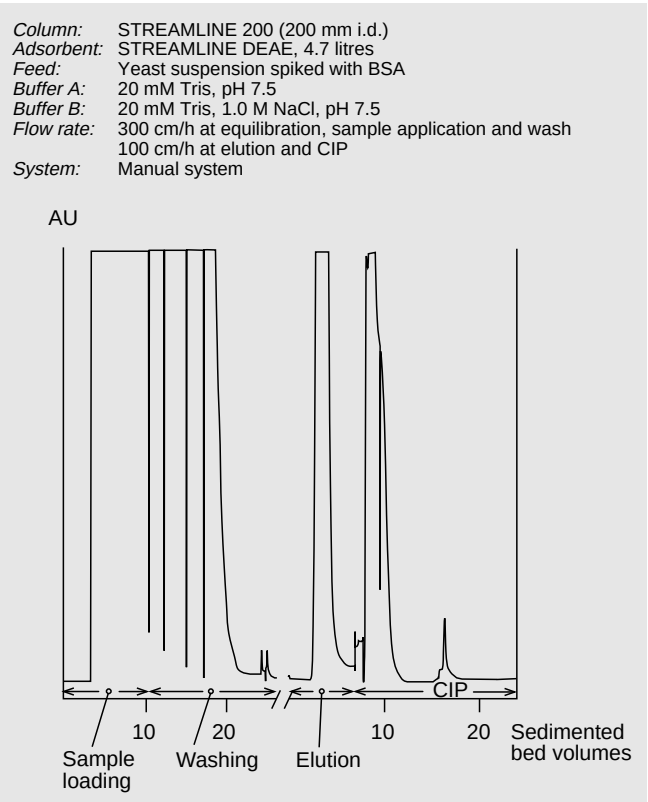


Fig. 5. Capture of BSA on STREAMLINE DEAE at pilot scale.

STREAMLINE 600 column

Two separate runs were performed on a STREAMLINE 600 column. The adsorbent was expanded and equilibrated by running 10 sedimented bed volumes of buffer A through the bed. At the start of expansion the adaptor was automatically lifted to a height of 85 cm. In order to record the height of the expanded bed at the end of the expansion and equilibration phase, the adaptor was automatically lowered to and kept just above the surface of the expanded bed by a programming sequence in the UNICORN method. This programming sequence lowers the adaptor when the adsorbent sensor indicates absence of adsorbent beads and lifts the adaptor when the sensor indicates presence of adsorbent beads.

At the start of sample application the adaptor was again lifted to a height of 85 cm. Reversal of flow direction during sample application, to prevent clogging of the column nets and packing of adsorbent beads under the adaptor end-plate, was automatically performed by another programming sequence in the UNICORN method. This sequence was triggered by a pressure increase to above 0.3 bar, as indicated by the pressure sensor in the system and would cause a short reversal of the flow to remove clogged material in the nets. The volume of feed applied corresponded to approximately 10 sedimented bed volumes.

A wash with buffer A followed feed application. During the wash, the adaptor was automatically lowered to and kept just above the surface of the expanded bed, as described above. In the first run, this function was programmed to be executed at the end of the wash phase, when the descending UV-signal reached a value of 2.0 AU. In the second run, it was programmed to be executed when the applied feed had left the expanded bed, i.e. at the beginning phase of the wash step. This change resulted in a significant reduction in consumption of wash buffer. The wash continued until the UV-signal was below 0.1 AU at which point the flow was automatically stopped for 10 minutes to allow the bed to sediment. The adaptor was then automatically lowered to the sedimented bed surface, compressing the bed until a back-pressure of 0.3 bar was obtained. The packed bed was washed with buffer A followed by elution of the product with buffer B. A cleaning procedure (CIP/SIP) was performed as previously described using upward flow in expanded bed mode. During the CIP/SIP procedure the adaptor was positioned at a height of 30 cm.

A chromatogram from the second run is shown in Fig. 6.

Results from the run on STREAMLINE 600 are summarized in Table 3.

Compared to the first run, buffer consumption during the wash decreased by 250 litres in the second run as a result of lowering the adaptor early in the procedure.

The product peak from each scale was collected and analysed by size exclusion chromatography on a Superdex® 200 HR 10/30 column (see Fig. 7). The chromatograms were integrated and the area of the albumin peak was used to calculate yield.

The chromatographic profiles from the different runs on the Superdex 200 HR 10/30 column were identical, which indicates consistency in chromatographic performance from laboratory scale on the STREAMLINE 25 column up to production scale on the STREAMLINE 600 column.

Column: STREAMLINE 600 (600 mm i.d.)
Adsorbent: STREAMLINE DEAE, 42 litres
Feed: Yeast suspension spiked with BSA
Buffer A: 20 mM Tris, pH 7.5
Buffer B: 20 mM Tris, 1.0 M NaCl, pH 7.5
Flow rate: 300 cm/h at equilibration, sample application and wash
100 cm/h at elution and CIP
System: STREAMLINE system/UNICORN

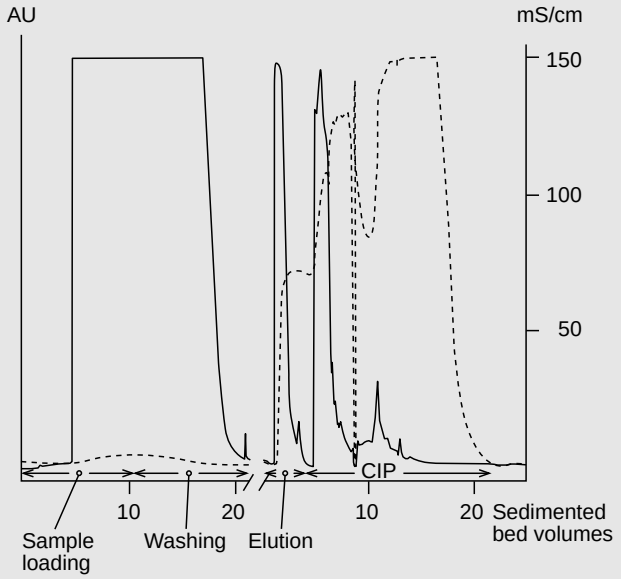


Fig. 6. Capture of BSA on STREAMLINE DEAE at production scale.

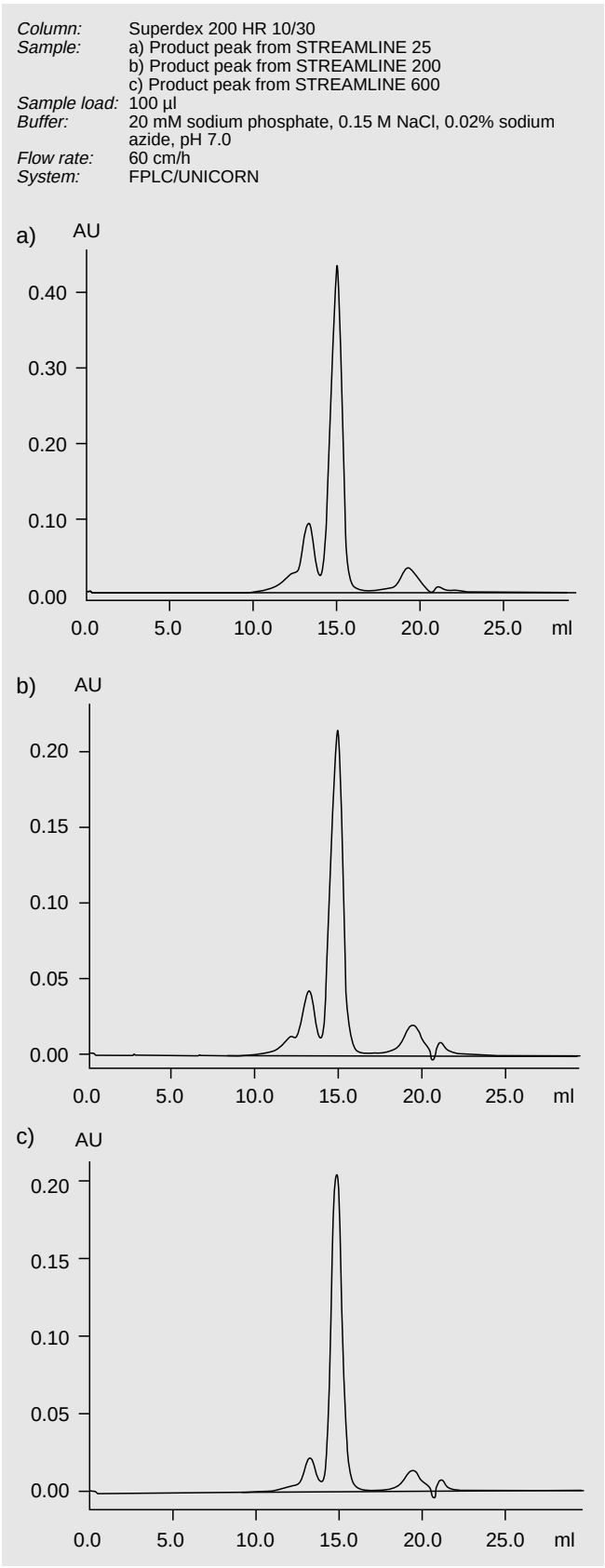


Fig. 7. Analytical gel filtration of product peaks from capture step on STREAMLINE DEAE.

Sanitization study

A sanitization study was performed on the STREAMLINE 600 column (containing 42 litres of STREAMLINE DEAE adsorbent) and STREAMLINE system used at full production scale. The purpose of the study was to evaluate the hygienic status of the complete production set-up and to verify the sanitization efficiency of the CIP/SIP protocol applied during the process runs.

Procedure

The study was designed to mimic a real expanded bed adsorption process. Two tests were performed, one with a recombinant yeast (*S. cerevisiae*) culture broth as feed material and one with a bacteria (*E. coli* K12) culture broth as feed material. Approximately 5 sedimented bed volumes of the yeast culture broth and 2.5 sedimented bed volumes of the bacteria culture broth were applied in two separate process runs. The concentration in the feed was 1×10^9 viable organisms/ml for the yeast and 1.2×10^{10} viable organisms/ml for the bacteria.

The feed was applied to the expanded bed at a flow rate of 300 cm/h followed by a wash with 50 mM NaCl until the UV-signal stabilized at the baseline level. After the wash, the bed was eluted with 4 sedimented bed volumes of 1 M NaCl. The elution was performed in sedimented bed mode with the adaptor positioned on top of the sedimented bed, using downward flow at a flow rate of 100 cm/h. After the elution step the bed was subjected to a CIP/SIP sequence in expanded bed mode using upward flow and with the adaptor positioned at twice the sedimented bed height. The CIP/SIP sequence consisted of 0.5 M NaOH/1 M NaCl (contact time 4 hours), buffer A (3 sedimented bed volumes; 100 cm/h), 20% ethanol/10% HAc (3 sedimented bed volumes; 100 cm/h) and buffer A (10 sedimented bed volumes; 100 cm/h).

At the end of the first CIP/SIP step the adaptor was raised to its upper position. The hydraulic chamber was then washed by pumping 0.5 M NaOH/1 M NaCl from the hydraulic pump. During this wash phase the adaptor was lowered and raised 3 times, 5 cm in each direction, and the flow was sequentially directed through all the different inlets to the hydraulic chamber. After cleaning the hydraulic chamber was refilled with hydraulic liquid and the adaptor lowered to twice the sedimented bed volume. The CIP/SIP sequence of the expanded bed was continued with the next solution.

During the test with yeast, it was necessary to include an additional step to remove all yeast cells from the air trap. This involved applying the 0.5 M NaOH/1 M NaCl solution at a higher flow rate through the system.

Results

The extent of microbial survival was determined by sampling the adsorbent, the hydraulic liquid in the column, eluate from the column, and a number of critical test points in the column and system, see Tables 4 and 5 and Fig. 1. The test points from the column and system were sampled with swabs. Samples were cultivated on TSA (Trypticase Soya Agar) plates incubated at 31 °C ±2 for 5 days.

No surviving organisms were detected in the adsorbent, the hydraulic liquid, column eluate, at any of the test points in STREAMLINE 600 column or in the system after infection with *E. coli* or *S. cerevisiae*, see Tables 4 and 5.

The results indicate that the proposed sanitization technique allows efficient sanitization of STREAMLINE 600 column and system.

Table 4. Location of test points and results from STREAMLINE 600 column.

Test point No.	Location	Results (CFU) *E. coli*	Results (CFU) *S. cerevisiae*
1	Adsorbent	0	0
2	Hydraulic liquid	0	0
3	Column eluate	0	0
4	Adaptor end piece	0	0
5	Adaptor distributor plate	0	0
6	Adaptor O-ring	0	0
7	Groove for adaptor O-ring	0	0
8	Bottom end piece	0	0
9	Bottom distributor plate	0	0
10	Sealing O-ring tube/bottom end piece	0	0
11	Hydraulic chamber	0	n.d.
12	Adaptor body above O-ring	0	n.d

CFU = colony forming units, n.d. not determined.

Table 5. Location of test points and results from sanitization study on STREAMLINE system.

Test point No.	Location	Results (CFU) *E. coli*	Results (CFU) *S. cerevisiae*
1	Priming valve before main pump	0	0
2	Diaphragm valve before air traps	0	0
3	Priming valve on air trap (sample)	0	0
4	Upper O-ring in air trap (sample)	0	0
5	Lower O-ring in air trap (sample)	0	0
6	Top lid of air trap (sample)	0	0
7	Bottom piece of air trap (sample)	0	0
8	Diaphragm valve at column inlet	0	0
9	Diaphragm valve at column outlet	0	0
10	Conductivity flow cell	0	0
11	Conductivity flow cell (gasket)	0	0

Conclusion

Scalability of STREAMLINE expanded bed adsorption has been proved by a process example optimized on STREAMLINE 25 column, verified at pilot scale on STREAMLINE 200 column and finally scaled up to full production scale on STREAMLINE 600 column.

Scalability has been verified by the consistency in yield and by the consistency of chromatographic performance as judged by the appearance of chromatographic curves and analytical gel filtration of collected product peaks. RTD testing and breakthrough capacity determination gave further evidence for consistency between the different scales.

The special features of large scale STREAMLINE columns, such as the adsorbent sensor and adaptor position indicator, together with UNICORN control system, make automated production scale runs possible.

The proposed sanitization technique was found to be efficient for removing microbial contamination from the column and system, indicating a high hygienic status of the production scale STREAMLINE set-up.

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Product information

STREAMLINE is a range of columns systems and media for expanded bed adsorption. Large scale columns, i.e. over 200 mm i.d. and systems are custom-designed. Media and small scale columns can be ordered through your local Pharmacia Biotech representative, please contact your representative for further information about any of the products used in this application and their Data Files.

Data File	Code No.
STREAMLINE DEAE	18-1111-73
Superdex 200 HR 10/30	18-1034-11
STREAMLINE 25	18-1112-02
STREAMLINE 200	18-1109-30
FPLC System	18-1103-42
BioPilot System	18-1033-44
UNICORN control system	18-1111-20

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